CHAPTER V

ORAL CONTRACEPTIVE AND THE METABOLISM OF GLYCOSAMINOGlyCANS AND GLYCOPROTEINS

Part I  Effect of administration of oral contraceptive (OC) on the concentration of aortic glucosaminoglycans (GAG) in experimental animals

Part II  Effect of administration of oral contraceptive (OC) on the concentration of carbohydrate components of aortic glycoproteins in experimental animals
Part I

Effect of administration of oral contraceptive (OC) on the concentration of aortic glucosaminoglycans (GAG) in experimental animals

Glycosaminoglycans (GAG) are present in tissues covalently linked to protein-polysaccharide complexes denoted as proteoglycans. These carbohydrate macromolecules are important components of the connective tissue and form a vital part of the cardiovascular structure. Connective tissue proteoglycans are distinguished from other mammalian carbohydrate protein complexes by the presence of relatively large polysaccharide chains containing repeating disaccharide units as their most characteristic feature. They are usually composed of hexosamine and uronic acid. Two uronic acids, D-glucuronic acid (GA) and iduronic acid, and two hexosamines, D-glucosamine and D-galactosamine are the constituent sugars of these polysaccharides. The amino group of the hexosamine is always substituted by either alkyl or sulphate group. Further the hydroxyl group may be substituted with ester sulphate. In addition several other monosaccharides may be
present as integral components including sialic acid, mannose, fucose, galactose or xylose. With the exception of galactose (in keratosulphate) these sugars are not part of the characteristic repeating disaccharides and occur as either on side branches or constituents of specific carbohydrate protein linkage region. Glycoprotein (GP) on the other hand contain lesser amounts of carbohydrates. They are proteins with covalently bound hexosamines, sialic acid and neutral monosaccharides but no uronic acid is present.

Dorfman\(^\text{245}\) has suggested that the GAG of the connective tissue have a role in a number of physiological and pathological processes including calcification, control of electrolytes and water in intracellular fluids, wound healing, lubrication and maintenance of the stable transport medium of the eye. The participation of acidic GAG is undoubtedly associated with their polyamine nature resulting from the presence of a large number of carboxyl and sulphate residues. One major responsibility is its response to an injury to produce an inflammatory reaction and subsequent repair. It is also apparent that the connective tissue components enter into genetic and immunological functions of the body. The multiplicity and importance of these roles indicate a basic function of connective tissue in the maintenance of the cardiovascular system. It is suggested
that the macromolecular substances present in the cardiovascular connective tissue are important from a genetic standpoint, are immunologically active, have in many cases enzymatic activity in cardiovascular diseases.

There are reports that after menopause, morbidity and mortality from atherosclerotic vascular diseases are more in women. Serum lipid changes have also been reported to occur coincident with the decrease in ovarian function in women but the possibility that estrogens may exert direct vascular effects was also postulated long ago. Hyperlipidemia has been reported to be one of the major risk factors in atherosclerosis. It has been shown that the hyperlipidemia has been induced by the administration of oral contraceptives in experimental animals\textsuperscript{234}. In atherosclerosis the metabolism of GAG is derranged in addition to that of lipids\textsuperscript{119,120}. The concentration of sulphated GAG was found to decrease significantly in the aorta of atheromatous rats after an initial increase in the early stages\textsuperscript{246,247}.

Wolensky\textsuperscript{248} has reported that ovariectomy induced acceleration of connective tissue accumulation in the thoracic aorta in rats which was prevented by estrogen administration. Priest et al.\textsuperscript{249}, Salvani et al.\textsuperscript{250} and Hastings et al.\textsuperscript{251} reported reduced uptake of labelled
sulphate in the presence of estadiol. Malinow et al.\textsuperscript{252} observed suppression of GAG synthesis by arteries on administration of exogenous estogen. Endo and Yosizava\textsuperscript{253} reported that the metabolism of hyaluronic acid, low sulphated chondroctin sulphate, heparin sulphate, chondritin sulphate A and chondrotin sulphate B was stimulated markedly by estrogens in the uterus of ovariectomised rabbits and an increase in the concentration of GAG was observed in estrogen treated ones. Kowalawaks\textsuperscript{254} reported increased incorporation of labelled sulphate in sulphated MPS of aorta on administration of estradiol. Kang Jeg Ho et al.\textsuperscript{255} failed to demonstrate any substantial change of aortic acid MPS on estrogen treatment. Apart from these, no other reports seem to available on the effect of administration of oral contraceptives and its components on the concentration of GAG. In view of this it was considered necessary to investigate the effect of oral contraceptives and its components separately to female rats on the metabolism of glycosaminoglycans (GAG).

Materials and Methods

Female ino rats (Sprague-Dawley strain, average weight 150 g) were divided into four groups of 12 rats each.
Group 1 - Control rats
Group 2 - Rats administered ovulen
Group 3 - Rats administered progestin
Group 4 - Rats administered estrogen

The composition of diet fed to animals was the same as described in Chapter III, Part I. The consumption of diet was adjusted to be the same for all the groups. The rats of group 2 were administered high dose OC namely Ovulen (10 μg of estrogen and 100 μg of progestin) and those of group 3 were given 10 μg of estrogen and that of group 4, 100 μg of progestin, orally through a tube for a period of six cycles. At the end of the experimental period the rats were sacrificed and aorta of the animals of the different groups were collected in the ice cold containers for the estimation of GAG. Details of the procedure used for the determination of total GAG are given in Chapter II.

Results

The diet consumption was similar in rats of all groups (11.2±2.0 g) but rats of group 2 showed higher in weight (32.2±1.6 g) for control rats & 37.6±1.3 g for group 2, 33.1±1.5 for group 3 and 34.6±1.2 g for group 4 respectively.
Concentration of the total GAG in aorta

Results are given in Table 55.
't' values are given in Table 55a.

The concentration of total GAG significantly decreased in experimental animals of group 2, 3 and 4 when compared with the control group.

Part II

Effect of administration of oral contraceptive (OC) on the concentration of carbohydrate components of aortic glycoproteins in experimental animals

Glycoproteins (GP) along with GAG are important carbohydrate macromolecules in the connective tissue. Glycoprotein are a family of complex proteins which covalently bound carbohydrates. They generally differ from glycosaminoglycans (GAG) in that they do not contain uronic acid and sulphate esters. However, the presence of sulphated glycoproteins have been reported\textsuperscript{256,257}. The monosaccharide constituents of glycoproteins are hexosamines in the acetyl form, neutral sugars - glucose, galactose, fucose, mannose from a number of sources like serum, glandular secretions, cystic fluids, urine etc. have been studied. In addition to
Table 55 Concentration of total GAG in Aorta
(expressed as ug uronic acid/g dry defatted tissue)

<table>
<thead>
<tr>
<th>Group</th>
<th>Aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6366 ± 200</td>
</tr>
<tr>
<td>2</td>
<td>4047 ± 140.4a</td>
</tr>
<tr>
<td>3</td>
<td>3112 ± 108.9a</td>
</tr>
<tr>
<td>4</td>
<td>3708 ± 128.6a</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for 6 rats. Group 2, 3 and 4 have been compared with group 1

\( a = P < 0.01; \ b = 0.01 < P < 0.05 \)

Table 55a 't' values for Table 55

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1 &amp; 2</th>
<th>1 &amp; 3</th>
<th>1 &amp; 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>9.0</td>
<td>14.8</td>
<td>11.2</td>
</tr>
</tbody>
</table>
soluble glycoproteins structural or insoluble glycoproteins from aorta have also been studied.

Soluble glycoproteins from connective tissue such as granulation tissue and aorta have been isolated by Berenson and co-workers and interesting complexity was observed when these preparations were subjected to polyacrylamide gel electrophoresis. These observations have led them to believe that a family of closely related glycoproteins occur in the cardiovascular connective tissue; certain glycoproteins vary from one individual to another and is likely that genetic variation may occur in their make up.

Proteoglycans, collagen and structural glycoproteins are major components of the connective tissue matrix and an alteration in metabolism particularly in the arterial wall has been shown to be associated with cardiovascular diseases. Very little work seems to have been done in studying the changes taking place on the metabolism of glycoproteins on administration of oral contraceptives. We studied the changes in the aortic glycoproteins in rats administered oral contraceptives and its components. The results of these investigations are discussed in this section.
Materials and Methods

Female albino rats (Sprague-Dawley strain, average weight 150 g) were divided into four groups of 12 rats each as follows:

- Group 1 - Control rats
- Group 2 - Ovulen administered rats
- Group 3 - Progestin administered rats
- Group 4 - Estrogen administered rats

The composition of diet was the same as given in Chapter III part I. The rats of Group 2 were administered high dose OC (10 μg of estrogen and 100 μg of progestin) Ovulen, rats of group 3, were administered 100 μg of progestin and of group 4, 10 μg of estrogen for a period of six cycles. The experimental animals at the end of treatment period were deprived of food overnight and were sacrificed. The aorta of the animals of different groups were collected in ice-cold containers for estimation of total hexose, fucose and sialic acid. The details of the procedure for these estimations are given in Chapter II.

Results

a) Concentration of total hexose in the aorta

Results are given in Table 56.
't' values are given in Table 56a.

Results obtained indicate that concentration of total hexose in aorta of experimental rats of group 2, remained unchanged and that of group 3, decreased significantly when compared with control group. On the other hand, the concentration of total hexose in aorta of group 4, increased considerably in comparison with group 1.

b) Concentration of fucose in aorta

Results are given in Table 57.

't' values are given in Table 57a.

The concentration of fucose in aorta of rats administered ovulen and estrogen increased significantly while it remained unaltered in rats of progestin treated group when compared with control group.

c) Concentration of sialic acid in aorta

Results are given in Table 58.

't' values in Table 58a.

The concentration of sialic acid in aorta of rats treated with OC, estrogen and progestin increased significantly in comparison with control group.
Table 56 Concentration of hexose GAG in Aorta  
(expressed as mg/g protein of dry defatted tissue)

<table>
<thead>
<tr>
<th>Group</th>
<th>Aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60.2 ± 1.9</td>
</tr>
<tr>
<td>2</td>
<td>57.5 ± 1.8</td>
</tr>
<tr>
<td>3</td>
<td>50.1 ± 1.6a</td>
</tr>
<tr>
<td>4</td>
<td>73.7 ± 2.5a</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for 6 rats. Group 2, 3 and 4 have been compared with group 1

a = P < 0.01; b = 0.01 < P < 0.05

Table 56a 't' values for Table 56

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1 &amp; 2</th>
<th>1 &amp; 3</th>
<th>1 &amp; 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>1.0</td>
<td>4.0</td>
<td>4.3</td>
</tr>
</tbody>
</table>
Table 57 Concentration of fucose in Aorta
(expressed as mg/g protein of dry defatted tissue)

<table>
<thead>
<tr>
<th>Group</th>
<th>Aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.90 ± 0.35</td>
</tr>
<tr>
<td>2</td>
<td>14.21 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>12.10 ± 0.39</td>
</tr>
<tr>
<td>4</td>
<td>14.5 ± 0.52&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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</table>

Footnotes same as in Table 56.

Table 57a 't' values for Table 57

<table>
<thead>
<tr>
<th>Tissue</th>
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<th>1 &amp; 3</th>
<th>1 &amp; 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>4.10</td>
<td>0.57</td>
<td>4.4</td>
</tr>
</tbody>
</table>
Table 58 Concentration of sialic acid in aorta

(expressed as mg/g protein of dry defatted tissue)

<table>
<thead>
<tr>
<th>Group</th>
<th>Aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.02 ± 0.46</td>
</tr>
<tr>
<td>2</td>
<td>27.47 ± 0.82a</td>
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<td>3</td>
<td>33.65 ± 0.94a</td>
</tr>
<tr>
<td>4</td>
<td>24.03 ± 0.76a</td>
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</tbody>
</table>

Footnotes same as in Table 56.

Table 58a 't' values for Table 58

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1 &amp; 2</th>
<th>1 &amp; 3</th>
<th>1 &amp; 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>6.9</td>
<td>13.1</td>
<td>4.71</td>
</tr>
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</table>
Discussion

The results now obtained indicate that the administration of ovulen and its components has significant effect on the concentration of GAG in the aorta. The carbohydrate components of aortic glycoproteins are also affected in the animals administered ovulen and its components. Ovulen generally causes a decrease in the concentration of GAG of the aorta when compared to control rats not receiving the OC. Administration of estrogen and progestin in the concentration present in the ovulen also causes a similar decrease in the aortic GAG. The decrease in the aortic GAG in the rats administered these substances is quite significant in the light of changes reported in the aortic GAG in the atherosclerotic rats. A decrease in many of the GAG fractions has been reported by several workers in atheromatous rats.261-263.

The aorta is particularly important both from the point of view of lipid accumulation in atherosclerosis and the high concentration of GAG present GAG exists in the arterial tissue covalently linked to the protein core to form macromolecules - Proteoglycans. The molecular chain network formed by the intercellular matrix acts as a filter to other macromolecules transported through it. Whether due to
hydrostatic bulk flow or diffusion the transport will be retarded. It has been demonstrated that the degree of retardation is dependent on the size of the molecule transported as well as the polysaccharide concentration \(264\). The presence of negatively charged carboxyl and sulphate groups in the GAG enables these compounds to bind molecules having a positive charge. Complex formation between lipoproteins and GAG have been observed \textit{in vitro} \(265,266\). The presence of GAG in the connective tissue of the sub endothelial space well result in entrapping of lipoproteins in this region because of the molecular sieving effect. Preventing the passage of lipoproteins further into the intimal region of the aorta. The steric exclusions \(267,268\) effect of GAG will also result in the increased concentration of lipoproteins in this region.

It has been observed that during early stages of atheroma in rats there is increase in many of the sulphated GAG of the aorta \(119\). The increased concentration of GAG in the aorta may result in increased interaction with plasma lipoprotein to form insoluble GAG-lipoprotein complexes which may be deposited in the aorta. When the concentration of GAG is decreased in the aorta in the later stages of atherosclerosis with consequent decrease in the molecular sieving effect, the lipoproteins of the plasma are permitted
across the inner lining of the aorta into the intimal region. The decreased concentration of GAG even though resulting in decreased binding of lipoproteins will also cause decreased transport of lipoproteins across the arterial wall resulting in the accumulation of lipoproteins in the tissues. Thus an optimal concentration of GAG is required in the arterial wall to maintain its integrity, the GAG playing a role in complexing with the lipoproteins and the transport of lipoproteins across the arterial wall.

The results now obtained indicate that the administration of ovulen and its components causes similar changes in the aortic GAG as observed in the aorta in the atheromatous rats. It has been observed that the ovulen and its estrogen in high concentration produce increase in the serum and aortic lipids similar to those observed in atherosclerotic rats. Thus ovulen and its estrogen component may contribute towards atherosclerosis affecting the metabolism of lipids and GAG. Unlike in the case of lipids where estrogen and progestin produce opposite effects; they produce similar changes in the aortic GAG.

Ovulen and its components also cause similar effect on the aorta in the case of carbohydrate components of glycoproteins. Increase in fucose and sialic acid and no significant alteration in total hexose are observed in the
aorta in rats administered ovulen. These results are also relevant in the light of changes in the aortic glycoproteins taking place in the atherosclerotic aorta. An increase in total hexose and fucose and decrease in sialic acid are observed in the aortic glycoproteins in atheromatous rats. An increase in the fucose of aortic glycoprotein is similar to that observed in atheromatous rats, but the changes in total hexose and sialic acid appear to be different. Estrogen alone causes increase in fucose as in the case of ovulen while progestin has no significant effect. In the case of aortic total hexose while estrogen alone cause an increase progestin causes decrease. The lack of significant alteration in the aorta in the rats administered ovulen may be due to the opposing effects of estrogen and progestin.

No previous reports appear to be available on the effects of oral contraceptives on the aortic GAG or GP. Thus the present results indicate that oral contraceptives like ovulen may promote atherosclerotic changes in the aorta by their effect on GAG and GP in addition to that on lipids.